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Comparison of 9 Methods for the Determination of Cholesterol

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Summary: Seven enzymatic procedures for the determination of cholesterol in serum were compared with the *Liebermann-Burchard-* and a gas-chromatographic method. Using a decision matrix all methods could be ranked according to reliability and practicability. With the exception of the cholesterol oxidase-coupled *Kageyama* principle and the *Liebermann-Burchard* procedure, all the other methods showed similar reliability.

Vergleich von 9 Methoden zur Bestimmung von Cholesterin

Zusammenfassung: Sieben enzymatische Verfahren zur Bestimmung der Cholesterin-Konzentration im Serum wurden mit der *Liebermann-Burchard-* und einer gaschromatographischen Methode verglichen. Mit Hilfe einer Entscheidungsmatrix wurde eine Wichtung von Ergebnissen und Praktikabilität versucht. Dabei zeigt sich, daß sich mit Ausnahme des mit Cholesterinoxidase gekoppelten *Kageyama-* und des *Liebermann-Burchard*-Prinzips die übrigen Methoden etwa gleich zuverlässig verhielten.

Introduction

For many years, cholesterol was determined chiefly by the *Liebermann-Burchard* and the *Zak* methods. Both procedures are prone to various interferences, which are summarized in several reviews (1–5). In addition, they use a strong acidic milieu, a disadvantage which is particularly relevant in mechanised analytical systems, because of corrosion.

After *Richmond* (6,7), in 1972, reported the isolation of a bacterial cholesterol oxidase, various procedures were soon developed for the determination of cholesterol with the aid of this enzyme. In the meantime, in many laboratories, the enzymatic methods have replaced the older procedures mentioned above. They appear to be more specific and better suited for mechanisation.

The various enzymatic methods differ with respect to the coupled indicator reaction (table 1). After hydrolysis of cholesterol esters in the presence of cholesterol esterase, cholesterol oxidase activates the oxidation of cholesterol to Δ^4-cholestenon. The resulting H_2O_2 is measured in most procedures.

For comparison, we selected 3 test kits based on *Trinder*'s reaction (8), but with differences in the reaction mixture, one test kit applying the *Hantzsch* reaction according to *Kageyama* (9), one test kit using the NADP-coupled aldehyde dehydrogenase reaction (10) and one test kit following the principle first described by *Harders & Helger* (11). In addition the *Liebermann-Burchard* procedure and a gas chromatographic method were included in this study. The last method was primarily used to investigate the accuracy of the other tests. In an earlier report we found, in accordance with *Allain* et al. (12), that the procedure of *Abell* et al. (13) often recommended for reference purposes, leads to cholesterol values which are approximately 10% higher than those determined with enzymatic methods.

Materials and Methods

Enzymatic methods

All enzymatic methods were performed with commercially available test kits. The abbreviations used and some relevant data are summarized in table 1. Concentrations of the various components are listed in table 2 only for the *Trinder* principle, because the test combinations chosen show significant differences. For further details, see the information distributed by the manufacturers. All test kits were used as supplied by industry without further modifications. With the *Kageyama* procedure the incubation period was extended to 75 minutes according to l.c. (32). The ABA 100 and Labtronic ES 25 system were set up as reported in table 3 and 4.

Liebermann-Burchard procedure

The *Liebermann-Burchard* reaction was performed with a SMA 12/60 from Technicon (Technicon GmbH, D-6368 Bad Vilbel)

0340-076X/79/0017-0553$02.00

Table 1: Methods for the determination of the cholesterol concentration

Method number	Abbreviation used	Principle	Source of reagents	Catalogue number of test kit	Lot number of test kit	Analytical system	Measuring wavelength (nm)	Incubation temperature (°C)	Incubation time (min)	Sample volume (µl)
1	4-Aminophenazone (PAP)	ChO + ChE[1]) and *Trinder*'s reaction (12)	BM[2])	172626	1277516	ES 25[8])	Hg 546	37	15	10
2	*Kageyama*	ChO + ChE and *Kageyama*'s principle (9)	BM[2])	124079	64382501	ES 25[8])	Hg 405	37	75	20
3	Merckotest	ChO + ChE $I^- \xrightarrow{H_2O_2} I_2$	Merck[3])	14350	7500272	ES 25[8])	Hg 365	25	30	10
4	Aldehyde dehydrogenase (AlDH)	ChO + ChE AlDH-method according to *Haeckel & Perlick* (10)	[4])			ES 25[8])	Hg 334	37	10	10
5	ABA	ChO + ChE AlDH-method according to *Haeckel & Perlick* (10)	[4])			ABA 100[5])	340/380	37	10	5
6	GENT	ChO + ChE and *Trinder*'s reaction	Abbott[5])	6095	220 A1177N	ABA 100[5])	500/600	37	10	5
7	AA	ChO + ChE Technicon and *Trinder*'s reaction (12)	Technicon[6])	T21-0690-54 T21-0888	1066102 1327125	AA II[6])	525	37	9	200
8	SMA	*Liebermann-Burchard*	Merz u. Dade[7])	–	–	SMA 12/60[6])	630	room temperature	9	2000
9	GC	Gas-chromatography	–	–	–	Research[9]) Gas Chromatograph 5750	–	–	–	200

[1]) Cholesterol oxidase + cholesterol esterase. [2]) BM, Boehringer Mannheim (D-6800 Mannheim). [3]) Merck AG (D-6100 Darmstadt) [4]) Reaction mixture was prepared according to l.c. (10). [5]) Abbott GmbH (D-6070 Langen) [6]) AA II, cartridge No. 170-106-01 and SMA 12/60, cartridge No. 157A058, Technicon GmbH (D-6368 Bad Vilbel). [7]) Merz und Dade GmbH (D-8000 München 50). [8]) Labtronic GmbH (D-6239 Vockenhausen) [9]) Hewlett Packard (D-7030 Böblingen).

Table 2: Contents of the assay mixture of test kits from various manufacturers using the *Trinder* reaction.

Method[1])	4-Aminophenazone	GENT	AA
Potassium phosphate, mmol/l	390	50	400
pH	7.7	6.7	7.2
Phenol, mmol/l	9.85	13.86	6.42
4-Aminophenazone, mmol/l	0.99	0.79	3.84
Methanol, mmol/l	920.4	–	967.5
Hydroxypolyethoxydodecane, %	0.2	–	–
Surfactant, %	–	–	3.9
Sodium cholate, mmol/l	–	2.97	–
Carbowax-6000, mmol/l	–	0.2	–
Peroxidase, U/l	39	27393	2260
Cholesterol oxidase, U/l	59	165	191
Cholesterol esterase, U/l	197	116	136

[1]) For explanation of abbreviations see table 1

using method No. N-24 a. The reagents were purchased from Merz und Dade (D-8000 Munich).

Gas chromatography

For gas chromatography we used the method of *Siekmann* et al (14). The derivatisation was found to be unnecessary, and was omitted. Sample preparation: 200 µl serum and 1000 µl KOH (0.5 mol/l) in ethanol were incubated 30 min at 56°C. After cooling to room temperature 6 ml cyclohexane were added. The mixture was vigorously shaken for 5 min and then centrifugated at 4000 r.p.m. (5 min). Four ml of the cyclohexane phase was evaporated and the residue dissolved in 100 µl + 1 ml) of which 2 µl were injected into a research gas chromatograph 5750 G equipped with a flame ionisation detector (Hewlett Packard, D-6000 Frankfurt/Main 56).

Table 3: Protocol of the ABA 100

	Method No. 5 ABA	Method No. 6 GENT
Temperature:	37°C	37°C
Mode:	FR	NORM
Time;	10 min	10 min
Reaction:	up/rate	up/endpoint
Filter:	340/380	500/600
Corousel revolutions:	2	2
Syringe plate:	1:101	1:101
Zero:	.000	.000
Calibrate:	.500	.500
Decimal point:	.000	.000

Conditions of the gas chromatograph:
glass column 115 cm, column temperature 240°C, stationary phase 3% OV-101 on Gas Chrom Q, 100–120 mesh (Applied

Table 4: Protocol of the Labtronic ES 25 System

	Method No. 1 4-Aminophenazone (PAP)	Method No. 2[1]) *Kageyama*	Method No. 3 Merckotest	Method No. 4[2]) Aldehyde dehydrogenase (AlDH)
Temperature	37° C	37° C	25° C	37° C
Filter	546	405	365	334
Computer				
– programme	No. 3	No. 4	No. 3	No. 4
– factor	22.1	33.9	8.69	16.67
Photometer	S	S	S	S
– factor	1.00	1.00	1.00	1.00
Pipetter station				
– position 1				
syringe, µl	2500		2500	2500
volume selected, µl	1000		2000	1000
velocity	10		7	10
– position 2				
syringe, µl	50		50	50
volume selected, µl	10		10	10
velocity	10		10	10

[1]) Pipetting was performed manually with Eppendorf pipets according to the manufacturer's instructions. This method uses a sample blank without cholesterol oxidase.
[2]) 2 measurements, the first before and the second 10 minutes after the addition of 20 µl cholesterol oxidase. Mixing was performed with a mini-mix (Vitatron GmbH, D-5000 Köln 60).

Science Lab. Inc., catalogue No. 12702, distributed by Serva International, D-6900 Heidelberg), carrier-gas N_2 (30 ml/min).

The cholesterol concentration of the unknown samples was calculated from the regression line of the peak heights of standard solutions.

Standard solutions and calibration factors

Results were calculated with the factor supplied by the manufacturers for method 1–3.

With method No. 4 the coefficient of absorbance $\epsilon_{334\ nm}$ = 6.18×10^6 cm^2/mol (16) was used. Unter these conditions the cholesterol standard (5.00 mmol/l) according to *Richmond* was recovered almost 100% ($\bar{x}$ = 5.04, coefficient of variation 1.64%, n = 28).

Method 5 and 6 were referred to a standard solution prepared according to *Richmond* (15): 1450.02 mg cholesterol (purity 99%) were dried and dissolved in 50 ml propanol-2, of which 20 ml were combined with 15 ml Triton X-100 and then evaporated at 70°C under vacuum. The residue is transferred into a 100 ml volumetric flask with 40 ml hot, bidistilled water and 5 × 7 ml 200 g/l albumin solution. At room temperature bidistilled water is added up to the 100 ml mark. One (or 2) ml of this stock solution are mixed with 2 (or 1) ml of 70 g/l albumin solution to give a solution containing 5 (or 10) mmol/l cholesterol. This working standard solution is stable at 4°C for one month. For the GC procedure cholesterol was dried and directly dissolved in ethyl acetate. No significant difference was found between the cholesterol used and standard material supplied by the National Bureau of Standards (Washington, D.C., USA).

The working standard was also used as a control in all other methods (except GC).

Method No. 7 was referred to Precilip.
Method No. 8 (*Liebermann-Burchard*, SMA 12/60) was referred to Hyland reference serum (Travenol, D-8000 Munich, catalogue No. HD 045–022, lot No. 3656N002BA). Under this condition a value of 4.59 ± 0.1 mmol/l (n = 28) was found in the cholesterol standard according to *Richmond*.

Interference study

The test for interference was similar to that proposed by *Staehler* et al. (17). Ten or 100 ml of a large serum pool were mixed with the substance of interest as indicated in table 9. These samples were analyzed in various series together with several control samples (no substance added). The results from the control samples were used to calculate the mean and the ± 3.s-range (s = standard deviation). Bilirubin interference was studied by dissolving 6 mg bilirubin (E. Merck AG, catalogue No. 24519) in 500 µl bidist. H_2O and 25 µl NaOH (1 mol/l). When the bilirubin was completely dissolved, albumin (70 g/l) was added to 2 ml. An aliquot (100 µl) of this solution was mixed with 1000 µl serum. Bilirubin was omitted in the control experiments.

Reagents

Propanol-2 (catalogue No. 9634), cholesterol (No. 24622), ethyl acetate (No. 9623) and cyclohexane (No. 2828) were purchased from E. Merck AG (D-6100 Darmstadt), Triton X-100 (No. 37238) from Serva (D-6900 Heidelberg), albumin (No. ORDH) from Behringwerke AG (D-3550 Marburg), concentrated acetic acid (No. 33209) from Riedel de Haen (D-3016 Seelze) and KOH in ethanol from Boehringer Mannheim (D-6800 Mannheim, catalogue No. 15916).

Control materials

Precilip (catalogue No. 125059) and Preciset (catalogue No. 125512) were purchased from Boehringer Mannheim (D-6800 Mannheim), Seronorm (catalogue No. NYCO 350053) from Dr. Molter GmbH (D-6900 Heidelberg).

Statistical methods

The results of the correlation study were computed by main component analysis (18, 19). Serum samples were randomly selected from patients of our hospital. Other statistical methods were used according to l.c (20).

Results

Imprecision

The GC and *Kageyama* methods had the highest imprecision from day to day (tab. 5), followed by the SMA procedure, whereas all enzymatic methods had

Table 5: Precision from day to day of the various methods for the determination of the cholesterol concentration.

Control material (Lot number)	Assigned value [mmol/l] (range)	4-Amino-phena-zone (PAP)	*Kage-yama*	Mercko-test	Alde-hyde dehydro-genase (AIDH)	ABA	GENT	AA	SMA	GC
Standard solution	5.00	5.03[4])	5.45	4.94	5.04	–	–	5.39	4.59	
		2.1	7.0	1.4	1.5			1.5	2.9	
		(28)	(28)	(28)	(28)			(28)	(28)	
Preciset (1067544)	3.88	4.07	4.25	4.06	4.17	4.16	4.23	3.96	3.48	4.20
		1.8	7.4	2.1	2.8	1.9	3.2	2.3	2.9	6.15
		(28)	(28)	(28)	(28)	(28)	(28)	(28)	(28)	(8)
Seronorm (128)	2.80[2])	2.25	2.41	1.81	2.25	2.28	2.39	2.14	2.64	2.26
	(2.49–3.11)	2.3	12.5	2.8	1.0	2.9	1.4	1.2	4.0	11.4
		(30)	(30)	(30)	(14)	(30)	(30)	(30)	(30)	(9)
Precilip (455 A)	3.54[1])	3.83	3.84	3.44	3.49	3.71	3.85	–	3.92	3.76
	(3.04–4.04)	3.1	8.4	2.5	2.6	2.2	2.0		3.0	14.2
	4.00[2])	(30)	(30)	(30)	(30)	(14)	(30)		(30)	(5)
	(3.44–4.56)									
Kontrollogen L (452 A)	3.51[1])	3.27	3.84	3.10	3.34	3.35	3.64	3.21	3.76	
	(2.84–4.03)	2.5	7.7	2.1	1.9	1.3	1.1	1.9	2.6	
	3.67[2])	(30)	(30)	(30)	(30)	(30)	(30)	(30)	(30)	
	(3.13–4.21)									
	3.41[3])									
	(3.02–3.80)									

[1]) for *Kageyama* method
[2]) for SMA-method
[3]) for 4-aminophenazone (PAP)-method
[4]) mean value, coefficient of variation (number of contributing values)

almost the same, good precision under the experimental conditions chosen, with a mean coefficient of variation of about 2.0%.

Accuracy

The relation between various cholesterol concentrations and the absorbance difference obtained (linearity-test) was checked with dilutions from the stock standard solution. No relevant deviation (< 1%) in the range investigated (up to 15 mmol/l) was seen with the 4-aminophenazone, Merck, aldehyde dehydrogenase and ABA method. The range of linearity was slightly reduced with the *Kageyama* (13 mmol/l), GENT (12 mmol/l), SMA (13 mmol/l), AA (10 mmol/l) and GC (12 mmol/l) method.

For the intermethod comparison the GC method was included in this study as a non-enzymatic procedure of relatively high specificity. The correlation data are summarized in table 6. All enzymatic procedures showed a similar correlation with the GC-method; the highest negative bias was obtained with the Merckotest method.

For graphical presentation of the correlation data the GC method is less suited because of its high imprecision (table 5). For this purpose we preferred the 4-aminophenazone method (method No. 1 in table 1) which is now mostly applied in clinical chemistry. Its correlation with the GC-method is shown in table 6 and figure 1. Under these conditions the 4-aminophenazone method correlated well (tab. 7, fig. 1) with the Merckotest, alde-

Table 6: The correlation between the GC method (x – values) and all other procedures (y_i-values) for the determination of the cholesterol concentration in sera from various patients. All figures were calculated by principle component analysis according to l.c. (18).

Method	a (intercept)	b (slope)	r	n (pairs of results)
4-Aminophenazone (PAP)	– 0.205	1.040	0.963	115
Kageyama	+ 0.211	1.045	0.875	115
Merckotest	– 0.435	1.039	0.952	115
Aldehyde dehydrogenase (AIDH)	– 0.313	1.048	0.946	115
ABA	– 0.339	1.057	0.939	115
GENT	+ 0.032	1.027	0.952	115
AA	– 0.042	1.007	0.953	115
SMA	+ 1.220	0.803	0.855	109

Table 7: The correlation between the 4-aminophenazone (PAP) method (x-values) and all other procedures (y_i-values) for the determination of the cholesterol concentration in sera from various patients. All figures were calculated by principle component analysis according to l.c. (18).

Method	$\bar{x}$	$\bar{y}$	a (intercept)	b (slope)	r	n
Kageyama	4.80	5.20	− 0.28	1.141	0.893	165
Merckotest	4.80	4.56	− 0.28	1.009	0.983	165
Aldehyde dehydrogenase (AlDH)	4.80	4.72	− 0.08	1.000	0.982	165
ABA	4.80	4.96	+ 0.17	0.997	0.981	165
GENT	4.80	4.73	− 0.18	1.023	0.975	164
AA	4.80	4.79	+ 0.15	0.967	0.988	165
SMA	4.80	5.09	+ 1.01	0.850	0.894	159
GC	4.75	4.76	+ 0.38	0.922	0.963	115

hyde dehydrogenase, ABA, GENT, AA and GC procedures. The correlation with the *Kageyama* and SMA cannot be accepted. With Merckotest the arithmetic mean for the results from all patients was lower than with the 4-aminophenazone method. The histogram in figure 2 shows that this effect is not simply due to a parallel shift of the whole distribution pattern by a constant negative bias.

In addition, with some control sera, the Merckotest led to the lowest mean values, whereas with standard solutions the recovery was comparable to those of the other enzymatic procedures.

The esterase reaction was not actually tested. However, the good correlation between the *Liebermann-Burchard* or the GC method with its alkaline saponification step, and the other procedures using the enzymatic hydrolysis, indicates that the esterase action may have been sufficient in all tests used. The differences observed with control materials (table 5) could be explained by the various esterase sources which are chosen by the manufacturers.

Interference from endogenous substances

Assuming that the GC method is not disturbed by elevated serum concentrations of triglycerides, hemoglobin or bilirubin, the results of this procedure were compared with all other methods. In figure 3 the difference (in mmol/l) between both methods was plotted against the serum concentration of bilirubin. In the case of an interference the regression line should be above or below the middle line and have a slope. The ± 3 standard deviation line was calculated from the results obtained with sera which looked clear and were not considered to be contaminated by one of the three endogenous substances.

No interference was noticed from hemoglobin (up to a concentration of 7 g/l), or from turbidity caused by triglycerides (up to 12 mmol/l) under the conditions reported in figure 3.

Zak (3) mentioned the potential reaction of bilirubin with peroxide which could lead to an underestimation in procedures using an indicator reaction for H_2O_2. However, a distinct disturbance by bilirubin was only noticed with the *Liebermann-Burchard* method (fig. 3). This overestimation of the cholesterol concentration in the presence of bilirubin is well known. In the presence of very high bilirubin levels added to 2 different sera (tab. 8), the 4-aminophenazone method appeared to slightly underestimate, and the Merck method to slightly overestimate, the cholesterol concentration.

Uric acid (up to 2000 µmol/l) did not influence the cholesterol value in the enzymatic procedures.

Interferences from exogenous substances

50 various substances which represent the most common used drugs and anticoagulants (17, 20) were added in high, but relevant doses to pooled sera from several patients (tab. 9).

The anticoagulants did not interfere with any of the methods. No interferences were observed with the *Kageyama* (No. 2), aldehyde dehydrogenase (No. 4) and *Liebermann-Burchard* method (No. 8). Interferences with the SMA and *Kageyama* procedures could have been masked by the higher imprecision of these methods. The other methods were submitted to a further study in which the concentrations of the interfering substances added were varied (table 10). Most often the cholesterol concentration was underestimated. With hostacyclin an overestimation was observed with the Merckotest procedure (table 9 + 10). The recovery of cholesterol in the presence of ascorbic acid and especially of α-methyldopa was higher with the Merckotest than with other methods using *Trinder*'s principle. The interference of ascorbic acid with *Trinder*'s reaction is already known (23).

Practicability

The *Liebermann-Burchard* method has the disadvantage that it uses a strong acidic reaction medium. The *Kageyama* procedure has the most pipetting steps. The

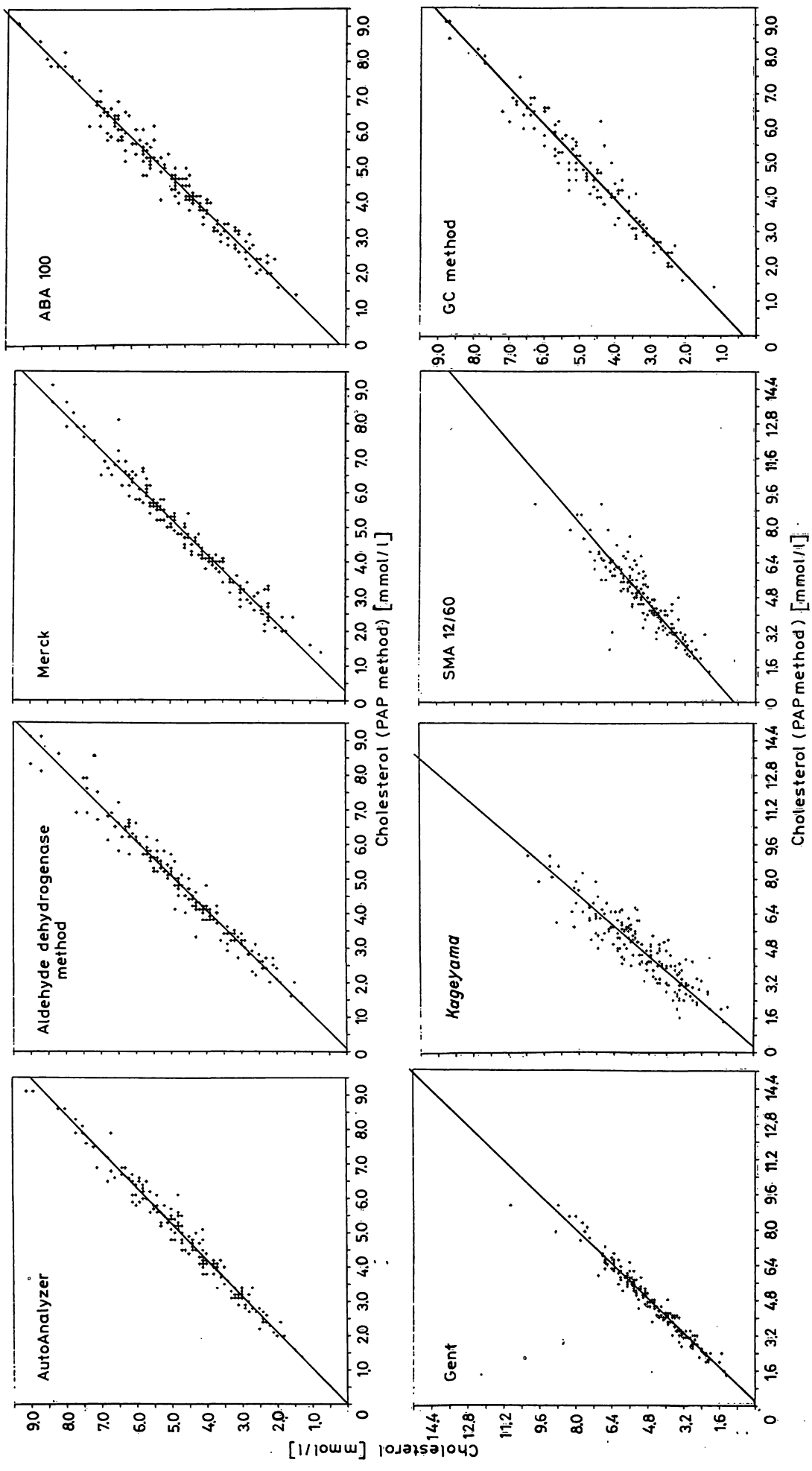

Fig. 1. Correlation between the 4-aminophenazone (PAP) method (abcissa) and all other procedures (ordinate) for the determination of the cholesterol concentration in sera from various hospital patients. Slope and intercept of the principle component were computed according to l.c. (16) and are reported in table 7.

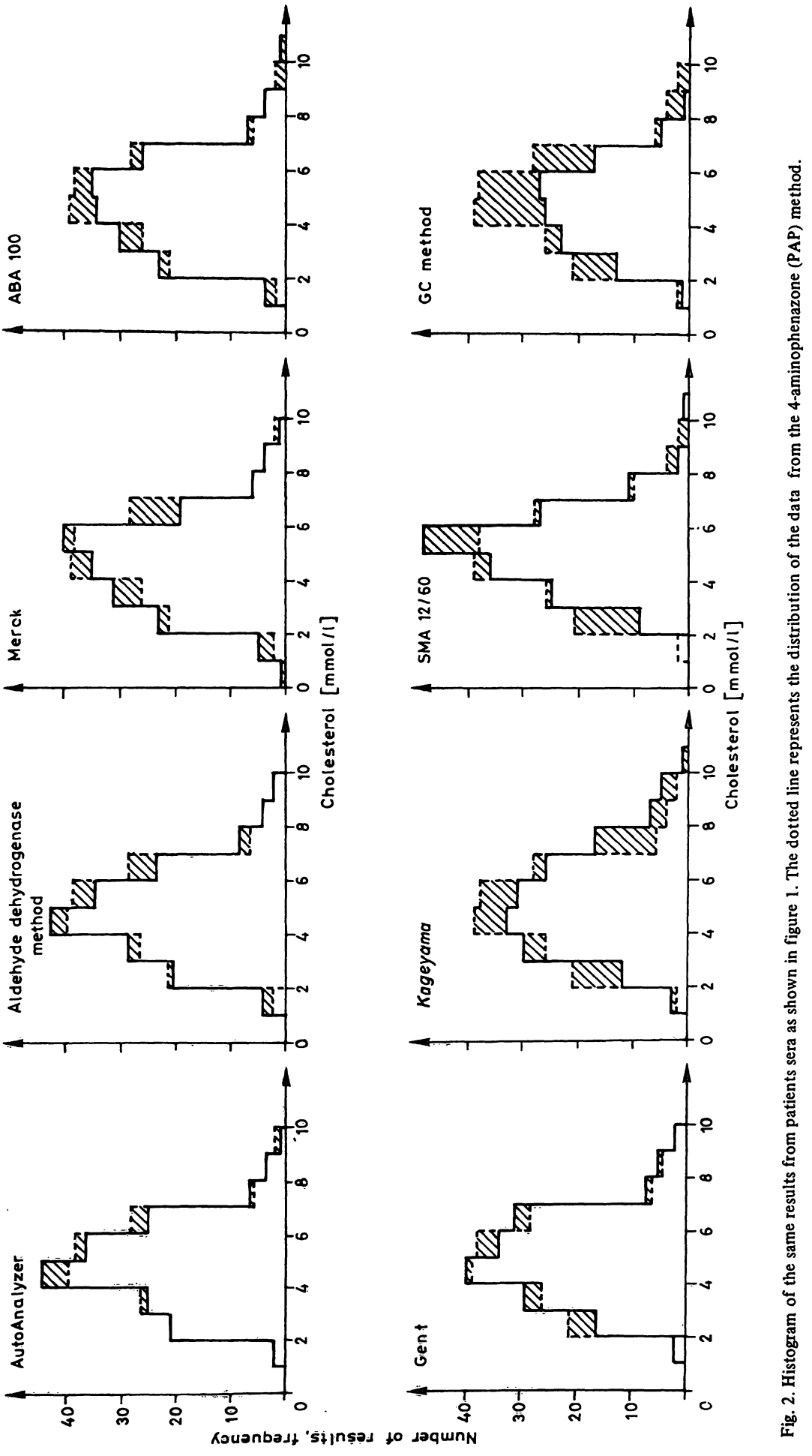

Fig. 2. Histogram of the same results from patients sera as shown in figure 1. The dotted line represents the distribution of the data from the 4-aminophenazone (PAP) method.

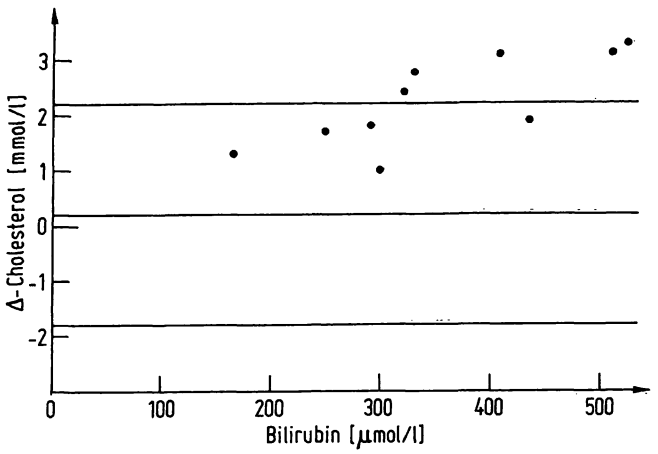

Fig. 3. Interference from bilirubin. The cholesterol concentration in various bilirubinemic sera was determined with the GC and the SMA methods. The difference (Δ cholesterol) between the GC and the other method is plotted on the ordinate. The upper and lower borderline were calculated as the threefold standard deviation from the results (Δ cholesterol) obtained with clear (non-bilirubinemic) sera (n, see table 7).

other methods, except for GC, are equally practicable, since all reagents can be combined into one mixture and cholesterol oxidase be used to start the reaction. In the aldehyde dehydrogenase method, the well known absorption coefficient of NAD(P)H can be used to calculate the result.

The aldehyde dehydrogenase method requires either a sample blank, a first absorbance reading before the reaction has been started, or a kinetic measurement, which is a disadvantage with manual procedures.

Conclusion

In table 11 a decision matrix is established by weighting the results reported above. From this all methods could be ranked. As expected the lowest rank was obtained with method No. 2 and 8.

Table 8: The influence of high bilirubin concentrations added to 2 different sera on various enzymatic procedures for the cholesterol determination. All figures are mean values (mmol/l) from 2 determinations.

Serum number	1	1		2	2	
Bilirubin, μmol/l	11	390	% of control	12	792	% of control
4-Aminophenazone (PAP)	3.64	3.50	96.1	3.60	3.10	86.1
Merck	3.63	3.78	104.1	3.28	3.50	106.7
ABA	3.90	3.87	99.2	3.62	3.61	99.7
Aldehyde dehydrogenase (AlDH)	3.90	3.88	99.5	3.54	3.55	100.3

Table 9: Recovery of cholesterol in human pooled sera containing various drugs. In the absence of any substance added a mean value and the range of 3 standard deviations was calculated from 15 determinations of the cholesterol concentration. Values outside this range are considered as due to interference (marked by an asterisk).

Trade name	I.N.N.[1])	Concentration (mg/l)	Cholesterol concentration (mmol/l)							
			PAP	*Kageyama*	Mercko-test	AlDH	ABA	GENT	AA	SMA
Amuno	indometacinum	30	5.35	5.83	5.22	5.34	5.38	5.70	5.68	6.6
Butazolidine	phenylbuta-zonum	280	5.37	4.75	5.20	5.34	5.38	5.73	5.75	6.8
Metalcaptase	*D*-penicil-laminum	960	5.12	6.54	4.68*	5.47	5.42	5.55	5.64	6.7
Prolixan	azopropazon-dihydrat	250	5.39	6.17	5.05	5.36	5.67	5.64	5.73	6.8
Resochin	chloroquinum	60	5.37	7.05	5.23	5.70	5.42	5.65	5.80	6.6
Tanderil	oxyphenbuta-zonum	600	5.48	4.71	5.01	5.58	5.42	5.49	5.55	6.9
Aponal	doxepinum	150	5.53	6.24	5.27	5.38	5.31	5.66	5.60	7.0
Megaphen	phenothiazinum	150	5.41	6.10	5.10	5.47	5.37	5.55	5.70	6.4
Multum	chlordiazepo-xidum	160	5.37	4.64	5.14	5.39	5.47	5.64	5.46	6.3
Aspirin	acidum acety-losalicylicum	750	5.39	6.03	5.15	5.26	5.53	5.70	5.68	7.0
Dolviran	acidum acetylo-salicylicum, etc.	780	5.69	4.98	5.14	5.24	5.40	5.75	5.64	5.7
Novalgin[2])	novaminsul-fonum	900	4.44*	6.13	1.05*	5.20	5.64	4.61*	5.21*	6.5
Benemid	probenecidum	260	5.61	5.70	5.25	5.38	5.33	5.70	5.68	6.7
Uriovac	benzbromaronum	60	5.66	5.09	5.24	5.38	5.40	5.62	5.70	6.5
Zyloric	allopurinolum	250	5.55	5.97	4.99	5.49	5.42	5.62	5.55	6.6

Table 9: (continued)

Trade name	I.N.N.[1])	Concentration (mg/l)	Cholesterol concentration (mmol/l)							
			PAP	*Kageyama*	Mercko-test	AlDH	ABA	GENT	AA	SMA
Angiografin	acidum triiod-benzoicum	2600	5.57	4.61	5.23	5.58	5.38	5.62	5.70	6.8
Biligrafin	adipinyltriiod-anilidum	1200	5.39	6.27	5.23	5.20	5.42	5.66	5.75	7.1
Urografin	acidum triiod-benzoicum	1500	5.08	4.81	5.19	5.68	5.47	5.59	5.69	6.6
Binotal 500	aminobenzyl-penicillinum	900	5.61	6.27	4.41*	5.39	5.47	5.68	5.67	7.3
Hostacyclin	tetracyclinum	200	5.57	4.64	5.69*	5.02	5.29	5.62	5.65	6.0
Paraxin	chloramphenicolum	600	5.50	6.71	5.06	5.30	5.49	5.60	5.68	6.5
Refobacin	gentamycinum	120	5.46	6.92	5.07	5.28	5.55	5.65	5.72	6.3
Buscopan	hyoscin-N-butyl-brominum	300	5.41	4.78	5.22	5.39	5.44	5.72	5.79	6.9
Cebion	acidum ascorbicum	400	3.01*	5.66	3.45*	5.30	5.56	4.03*	3.16*	7.5
Polybion	Vitamin B complex	12.9	5.35	6.00	5.20	5.13	5.44	5.64	5.67	6.0
Dipar	phenylethyl-biguanide	220	5.75	5.59	5.23	5.24	5.38	5.73	5.63	5.9
Euglucon 5	glibenclamidum	32	5.44	5.36	5.14	5.22	5.38	5.70	5.49	6.2
Rastinon	tolbutamidum	480	5.22	4.71	4.97	5.45	5.44	5.63	5.71	6.8
Dulcolax	bisacodylum	40	5.70	4.88	5.13	5.30	5.38	5.77	5.71	6.0
Durenat	sulfanilamido-pyrimidinum	231	5.72	5.02	5.13	5.07	5.36	5.73	5.65	6.2
Endoxan	cyclophosphami-dum	240	5.68	5.59	5.23	5.22	5.36	5.73	5.72	5.8
Methotrexat	acidum methyl-pteroylglutami-nicum	500	5.59	6.13	5.08	5.32	5.39	5.58	5.68	6.5
Intensain	carbocromenum	900	5.44	4.88	5.17	5.45	5.42	5.73	5.63	6.0
Furadantin	nitrofurantoinum	98	5.26	4.37	5.17	5.24	5.47	5.73	5.57	5.6
Lanicor	digoxinum	0.1	5.26	5.25	5.17	5.41	5.47	5.73	5.54	6.0
Lasix	furosemidum	60	5.35	4.95	5.12	5.68	5.40	5.65	5.47	5.7
Luminal	acidum phenyl-aethylbarbituricum	352	5.37	5.42	5.13	5.36	5.42	5.64	5.54	6.6
Macrodex 6%	dextranum 6%	6000	5.62	6.38	5.17	5.32	5.37	5.59	5.51	5.5
Modenol	thiabutazide, etc.	440	5.39	6.44	5.11	5.38	5.42	5.53	5.67	7.1
Presinol	methyldopa	400	3.05*	6.61	4.51*	5.53	5.48	3.72*	3.00*	7.0
Nicobion	nicotinamidum	40	5.50	6.24	5.09	5.30	5.42	5.71	5.68	6.3
Novadral	norfenefrinum	2.4	5.17	5.72	5.19	5.39	5.46	5.65	5.67	6.5
Solu-Decortin	prednisolonum	8	5.24	6.10	5.23	5.58	5.48	5.65	5.68	6.6
Marcumar	phenprocoumo-num	80	5.37	6.03	5.13	5.41	5.37	5.51	5.63	6.3
Na-Citrat	Na-citrate	5000	5.24	5.76	5.20	5.32	5.42	5.60	5.61	7.3
Liquemin	Na-heparinat	750	5.38	6.24	4.98	5.55	5.37	5.60	5.65	7.1
Na-Fluorid	Na-fluoride	2000	5.41	6.58	4.95	5.47	5.42	5.63	5.61	6.7
Na-Oxalat	Na-oxalate	2000	5.37	7.12	5.10	5.43	5.40	5.64	5.57	7.3
EDTA	Titriplex III	1000	5.53	6.47	5.20	5.02	5.40	5.73	5.63	6.8
Aldactone	spirolactonum	20	5.57	6.10	5.24	5.39	5.29	5.73	5.68	6.6

[1]) International non-proprietary names as proposed by the WHO (21).
[2]) Data are not considered in table 10, since clinically not relevant. This substance is rapidly metabolized and cannot be measured in significant blood concentrations (25).

Table 10: Interference in enzymatic procedures for the determination of cholesterol by varying concentrations of certain substances.

Method		PAP	Merckotest	GENT	AA
Interfering Substance	Concentration in the sample (mg/l)	Cholesterol (mmol/l)			
Ascorbic acid up to 0.7 mmol/l[1])	0	5.42	4.97	5.80	5.60
(overdosage up to 1.4) (24)	50 (0.28 mmol/l)	4.95	4.79	5.53	5.23
	100 (0.57 mmol/l)	4.38	4.48	5.19	4.76
	200 (1.14 mmol/l)	4.33	4.37	5.09	4.67
	300 (1.70 mmol/l)	4.04	4.13	4.89	4.40
	400 (2.27 mmol/l)	3.14	3.49	4.24	3.68
Presinol up to 193 mg/l[1]) (26)	0	5.42	4.97	5.80	5.60
	50	5.06	4.94	5.49	5.16
	100	4.69	4.87	5.13	4.80
	200	3.96	4.54	4.45	4.03
	300	3.18	4.27	3.63	3.26
	400	2.72	4.24	3.18	2.78
Binotal up to 300 mg/l[1])	0		4.97		
(28,29)	200		4.42		
	400		3.45		
	600		2.64		
	900		2.27		
Hostacyclin up to 8 mg/l[1])	0		5.42		
(28,29)	5		5.42		
	10		5.46		
	20		5.49		
	50		5.65		
	200		5.98		
Metalcaptase up to 10 mg/l[1])	0		5.42		
(30,31)	10		5.36		
	50		4.54		
	100		4.64		
	500		3.99		

[1]) Serum concentration under therapeutic conditions.

Table 11: Comparison of the various methods by weighting the quantitative data from table 3–6 and figure 3.

Method	1 PAP	2 *Kageyama*	3 Mercko-test	4 AIDH	5 ABA	6 GENT	7 AA	8 SMA
Precision[1])	++	–	++	++	++	++	++	+
Correlation[2])	+	–	+	+	+	+	+	–
Linearity	++	+	++	++	++	+	+	+
Accuracy[3])	++	+	+	++	++	++	++	–
Lack of interference from bilirubin	+	+	+	+	+	+	+	–
Lack of interferences from exogenous substances[4])	++	(+++)	++	+++	+++	++	++	(+++)
Practicability	+++	+	+++	++	+++	+++	+++	++
Sum of plus signs	13	7	12	13	14	12	12	7

[1]) Coefficient of variation < 3% ++, < 5% +, > 5% –
[2]) r^2 > 95.0% +, < 95.0% – (coefficient of determination, taken from l.c. (18))
[3]) estimated from table 5, 6 and 7
[4]) Table 9: no interference +++, interferences from ⩽ 3 substances ++, from more than 3 substances +

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